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Does agricultural food provide a good alternative to a natural diet for body store deposition in geese?

G. EICHHORN,^{1,2,3,†} H. A. J. MEIJER,⁴ K. OOSTERBEEK,⁵ AND M. KLAASSEN^{6,7}

¹Université de Strasbourg, Institut Pluridisciplinaire H. Curien — Département Ecologie, Physiologie et Ethologie, 23 rue Becquerel 67087 Strasbourg, France

²Centre National de la Recherche Scientifique, UMR7178, 67037 Strasbourg, France

³Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, The Netherlands

⁴Centre for Isotope Research, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands

⁵SOVON, Dutch Centre for Field Ornithology, P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands

⁶Centre for Integrative Ecology, Deakin University, Geelong, Victoria 3217, Australia

⁷Department of Animal Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700 AB Wageningen, The Netherlands

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Abstract. Over the past decades most goose populations have become increasingly dependent on agricultural crops during wintering and migration periods. The suitability of agricultural crops to support all nutritional requirements of migratory geese for the deposition of body stores has been questioned; feeding on agricultural crops may yield higher rates of fat deposition at the cost of reduced protein accretion due to an unbalanced diet. We compared amino-acid composition of forage, and investigated food-habitat use and dynamics and composition of body stores deposited by barnacle geese feeding on agricultural pasture and in natural salt marsh during spring migratory preparation. Overall content and composition of amino acids was similar among forage from both habitats and appeared equally suitable for protein accretion. There was no relationship between body composition of geese and their preferred food habitat. Fat and wet protein contributed with 67% and 33%, respectively, to body stores gained at a rate of 11 g/d throughout the one-month study period. We found no evidence of impaired protein accretion in geese using agricultural grassland compared to natural salt marsh. Our study supports the hypothesis that the expansion of feeding habitat by including agricultural grassland has played an important role in the recent growth of the East Atlantic flyway population of barnacle geese and other herbivorous waterbirds. Feeding refuges of improved grassland provide geese with an adequate diet for the deposition of body stores crucial for spring migration and subsequent reproduction, thereby alleviating the conflict with agriculture.

Key words: agriculture; amino acid profile; body composition; body stores; *Branta leucopsis*; global change; grassland; habitat shift; herbivore; migration; stable isotopes; Wadden Sea.

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† **E-mail:** goetz.eichhorn@c-strasbourg.fr

INTRODUCTION

Over the past decades most goose populations of the northern hemisphere have become heavily

dependent on agricultural crops during large parts of their wintering and staging periods, while goose numbers increased notably over the same period (Abraham et al. 2005, Fox et al. 2005,

Gauthier et al. 2005, Van Eerden et al. 2005). Determining the causes for these population increases is complex because of the parallel change of several other factors also known to enhance survival and/or recruitment, such as milder winters and reduced human persecution (Ebbinge 1991, Francis et al. 1992, Gauthier et al. 2005, Kery et al. 2006). Therefore, to ascertain the role of an increased use of agricultural food for the population dynamics of geese necessitates detailed studies on the nutritional ecology of geese. First findings indicate that geese attain higher energy intakes when utilizing agricultural crops compared to natural foods, especially when feeding on crops of (spilled) maize and other grains (McLandress and Raveling 1981, Madsen 1985, Alisauskas and Ankney 1992, Jónsson and Afton 2006), which are a highly digestible, carbohydrate-concentrated food source.

Preparation for long-distance migration requires the deposition of significant amounts of protein beside fat (Jenni and Jenni-Eiermann 1998). It has been argued for both barnacle *Branta leucopsis* and brent goose *Branta b. bernicla* that intensively managed grassland fails in providing geese with the necessary nutrients for an adequate deposition of protein, which natural grassland, as their traditional feeding habitat, does provide these nutrients (Prop and Black 1998, Prop and Spaans 2004). In both field studies on spring-staging geese, feeding in agricultural grassland and coastal salt marsh composition of accumulated body stores was inferred from nutrient budgets and forage digestibility. Notably lower nitrogen retention efficiencies were found in geese feeding on agricultural grassland, leading to lower protein deposition rates. It was suggested that these were caused by a mismatch between the amino acid composition of crop plants and the amino acid profile required by wild geese accumulating body stores. Their findings gave rise to the present study, where we compare forage in the two contrasting habitats in more detail, and estimate composition of accumulated body stores by an alternative, more direct, approach. The question whether artificial grassland can or cannot provide all of the nutrients geese usually gain from their natural feeding habitat has important management implications. Grazing

by wild geese can cause large reductions in the yields of agricultural grasslands (Percival and Houston 1992). Management of improved grassland as refuge for wild geese has therefore become a widely used tool in alleviating the conflict with agriculture (Vickery and Gill 1999, Patterson and Fuchs 2001). However, knowledge about the nutritional consequences for geese associated with this large scale shift towards and reliance on agricultural feeding is largely lacking.

In our study we focused on adult female geese because of their higher nutritional demands during reproduction as compared to males, and the known link between reproductive prospects and pre-migratory body condition in females (Ebbinge and Spaans 1995, Drent et al. 2007, Eichhorn et al. 2010). First, we evaluate amino acid profiles of the principal food plants used by barnacle and brent geese during their spring staging period in the Dutch Wadden Sea while feeding in two contrasting habitats: intensively farmed pasture land and natural coastal salt marsh (further referred to as 'pasture' and 'salt marsh'). Second, taking advantage of contrasting isotopic signatures of forage from these habitats, we investigate blood isotopic signatures of barnacle geese to infer their relative habitat use as well as mixing of diets from both habitats. Third, we examine possible food-habitat effects on body mass gain rates and body composition in barnacle geese, and address methodological issues for the estimation of protein deposition based on nitrogen budgets, highlighting the need to account for sources of non-protein nitrogen. Finally, we discuss changes in fuelling conditions and in spring migratory strategy of the barnacle goose as observed over the past decades.

METHODS

Study area and sampling of geese

The study was conducted on the Dutch Wadden Sea island Schiermonnikoog (53°30' N, 6°10' E) during spring staging (March to mid May, years 2002–2006) when several thousand barnacle geese and brent geese were present in two principal habitats: intensively managed pasture land and natural salt marsh (for a description of the study site see Van der Jeugd et al. 2001, Bos and Stahl 2003). The nearly sole

food plant available in pasture land was *Lolium perenne* (perennial ryegrass); amounts of *Poa pratensis* were negligible. For barnacle geese in the salt marsh the staple diet is composed of *Festuca rubra* and *Juncus gerardi*, with *Festuca* alone contributing ca. 90% (Van der Wal et al. 1998). Brent goose diet in the salt marsh consists mainly of *Puccinellia maritima* (70–80%) supplemented by *Festuca*, *Plantago maritima* and *Triglochin maritima* (Prop and Deerenberg 1991). Nomenclature of food plants follows Van der Meijden (1996). Forage plants, barnacle geese and their droppings were sampled from pasture and, at ca. six kilometers distance, from unfarmed salt marsh in the eastern part of the island.

A total of 109 adult (>1 year) female barnacle geese (as determined from absence of juvenile feathers and cloacal sexing) were trapped by cannon-netting at five occasions during spring 2004: in salt marsh on 18 March and 14 April and in pasture on 1, 16 and 20 April. Birds were weighed (± 1 g) with a platform scale and measured for tarsus length (± 0.1 mm), with calipers and head and wing length (± 1 mm) with a ruler. In a randomly selected sub-sample of 54 birds body composition was determined by means of isotope dilution (Speakman et al. 2001). Birds were injected intra-abdominally with a 99.9% deuterium isotope solution (Sigma Chemicals) using a 1.0 ml insulin syringe (equivalent to 1.1153 ± 0.0021 SD g, $N = 20$) and sampled for blood from the brachial vein 90 min later. For an estimate of average deuterium background level additional blood samples were taken on 5 birds (prior to isotope administration) at 3 trapping occasions. Time between trapping and start of isotope dilution measurements was normally one hour or more, when geese had already largely emptied their guts. Samples were stored in flame sealed micro-capillaries. Birds were kept in cages with no access to food and water during the isotope dilution measurements. An equilibrium time of 90 min was found sufficient to allow for adequate mixing of the marker solution with the body water pool (Eichhorn and Visser 2008). Additional blood samples were taken from another sub-sample of 34 birds (despite equal efforts in both habitats, 26 caught in agricultural pasture and 8 caught in salt marsh) overlapping in 31 birds with the sample

measured for body composition. The blood was centrifuged and divided into plasma and cells which were stored in a freezer for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic measurements (see below). The experimental protocol was approved by the animal experimentation committee of the University of Groningen, license DEC 4081A.

Evaluating food amino acid profiles

For evaluating food plant quality with respect to protein deposition we used the ideal amino acid ratios concept which states that amino acids must be digested in certain proportions, depending on actual requirements, to maximize the efficiency of incorporation into body protein (Klasing 1998). Most relevant here are the essential amino acids which must be supplied by the diet. Using this concept, the proportions of essential amino acids are expressed relative to lysine, which is regarded the first limiting amino acid in most plant based diets for animals during intensive protein accretion (Baker 2003). In the absence of ideal amino acid ratios for geese we relied on data established for growing chicken by Baker (2003). Although this demands cautious interpretation, we nevertheless believe it represents an informative approach since the amino acid composition of the same tissues across different species, including galliforms and anseriforms, is similar (Murphy 1994) and amino acid requirements for the growing chicken appear similar to those for growing ducks and goslings (Klasing 1998: Table 6.1). Six food plant species utilized by spring staging barnacle geese and brent geese on Schiermonnikoog (see above) were sampled for amino acid analysis between 1 and 4 May in both 2004 and 2005. Amino acid contents were standardized to a dry matter content of 100% and averaged over the two samples per species. Nitrogen and amino acid analyses were conducted by DEGUSSA Amino-LAB, Germany.

Isotopic signatures of diet and blood

The two studied habitats differ in food plant composition and agricultural practice (unfarmed marsh versus highly fertilized pasture), leading to distinct isotopic signatures in goose forage. We determined isotopic patterns in samples of barnacle goose droppings collected in salt marsh ($N = 21$) and agricultural pasture ($N = 15$) during

their spring-staging in 2002, 2003, 2004 and 2006. Each sample consisted of at least 10 droppings. Retention time of food in the digestive tract was 2.3 hours during staging in April (Prop and Vulink 1992). Therefore, care was taken to collect only fresh droppings produced from geese which spent at least three hours in the local feeding habitat. Dropping samples are suitable because more than 60% of ingested food leaves the gut undigested (Prop and Vulink 1992) and they provide a representative isotopic signature of the diet mix. Moreover, we found good correlation between isotopic signatures of droppings and the respective food plants (G. Eichhorn, *unpublished data*). Isotopic signatures of droppings, blood plasma and blood cells were used to estimate dietary input from pasture and salt marsh habitat over different time frames. Blood plasma isotopic signatures reflect diet choice over a period of about a week, whereas the lower turnover of blood cells makes them representative of diet choice over 1 to 2 months (Klaassen et al. 2010). Samples of droppings, blood plasma and blood cells were analyzed for $\delta^{15}\text{N}$ (‰ difference from the $^{15}\text{N}/^{14}\text{N}$ ratio in atmospheric N_2) and for $\delta^{13}\text{C}$ (‰ difference from $^{13}\text{C}/^{12}\text{C}$ ratio in Vienna PeeDee limestone) in a HEKAtech EuroEA elemental analyzer coupled on-line through a Finnigan ConFlo interface to a Finnigan Delta S isotope ratio mass spectrometer. Reproducibility based on replicate measurements of a casein standard ($N = 144$) during the period of measurements was 0.14‰ (=SD) for $\delta^{15}\text{N}$ and 0.13‰ (=SD) for $\delta^{13}\text{C}$.

Analyses of nitrogen compounds in food and droppings

Analysis of nitrate content in food plants and fecal and urinary nitrogen in bird droppings were conducted at the Chemical and Endocrinology Laboratory, Animal Science Group, University of Wageningen, The Netherlands. Total nitrogen in bird droppings contains fecal nitrogen (mainly originating from undigested food) and nitrogen from urinary waste products. Fecal nitrogen was determined after Terpstra and De Hart (1974) and subtracted from total nitrogen of droppings to yield urinary nitrogen.

Body composition analysis from isotope dilution

The procedures for determination of $\delta^2\text{H}$ in blood samples and estimates of body composition (Eichhorn and Visser 2008) are given in brief here. The blood in the capillary tubes was distilled in a vacuum line. The actual $\delta^2\text{H}$ measurements were performed in automatic batches using a Hekatech high-temperature pyrolysis unit coupled on-line to a GVI IsoPrime isotope ratio mass spectrometer for the actual isotope analysis. At least three internal water standards chosen to cover the entire enrichment range of the blood samples were prepared and analyzed following the same methods. All sample analyses were run at least in duplicate, more times if values differed by more than 2.5%, and we used the average of values differing from each other by less than 2.5%. Total body water determined from the dilution space (TBWd) was calculated using dose quantity (Q_d , mol), dose ^2H concentration (C_d , atom %), background ^2H (C_b , atom %), and ^2H concentration of individual blood samples (C_i , atom %): $\text{TBWd} = 18.02 \times Q_d \times (C_d - C_i)/(C_i - C_b)$.

In general, TBWd systematically overestimates TBW. Eichhorn and Visser (2008) found this overestimation in the barnacle goose to be 7%, and also found the general relation $\text{TBW} = 96.034 + 0.852 \times \text{TBWd}$ ($r^2 = 0.976$, $P < 0.001$), that we use here as well. Assuming a water content in the fat-free mass of waterfowl of 66.6% (Eichhorn and Visser 2008), fat-free mass (FFM) was calculated as $\text{FFM} = \text{TBW}/0.666$ and fat mass (FM) was subsequently inferred from body mass (BM) as $\text{FM} = \text{BM} - \text{FFM}$. Variation of FFM over the study period was further equated to variation of wet protein mass, assuming that the contribution of other constituents (carbohydrates and minerals) was negligible. Eichhorn and Visser (2008) evaluated the accuracy of this method for barnacle geese ($N = 21$) ranging in FM from 37 to 540 g and found an absolute error of predicted FFM or FM (calculated as deviations of predicted from observed values) of 22 ± 26 g (mean \pm SE).

Data analysis

We used Bayesian stable isotope mixing models to estimate contributions of pasture and salt marsh forage to the diet of barnacle geese as inferred from their blood plasma and blood cell

signatures using MixSIR (Semmens and Moore 2008), running all models with uninformative priors. Diet-tissue fractionation factors (mean \pm SD) as reported for herbivorous waterfowl by Hahn et al. (2012) were used: 4.4‰ and 0.3‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in plasma, respectively; 3.6‰ and -0.5‰ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in blood cells, respectively. The number of posterior draws in MixSIR was always above 67880. Models were run for groups (i.e., catches) of geese and, in order to relate diet choice directly to body composition, for individual birds.

The first principal component (PC1) generated from a principal component analysis including tarsus, head and wing length explained 66% of total variance of these three body structural measurements, and was used to account for variation in body size when modeling variations in BM, FFM and FM of barnacle geese. Multivariate general linear models (MANOVA, MANCOVA) were used to test for habitat effects on the combined response in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ or FFM and FM, respectively. Significance in multivariate tests was evaluated using Pillai's trace. Univariate general linear models (ANCOVA) were built to further estimate gain rates of BM, FFM and FM during the study period. Main effects and all possible two-way interactions were tested and non-significant terms were removed by backward deletion from the models. Assumption of univariate normality within groups was checked using Shapiro-Wilkes test. This was also used for multivariate models, because univariate normality is a precondition, although it does not guarantee multivariate normality. Equality of variances between groups was checked using Levene's test, and the assumption of homogeneity of covariance matrices for multivariate tests was checked using Box's test. Statistical analyses were performed in SPSS, version 18. Statistical significance was inferred at $P < 0.05$.

RESULTS

Differences in amino acid contents were small and their relative proportions were similar among food plants from both habitats (Fig. 1). Higher nitrogen contents in *Triglochin*, *Puccinellia* and *Lolium* were reflected in higher amino acid contents, and at similar proportions so for most of the amino acids but proline being a notable

exception. When contrasted to the assumed ideal amino acid pattern, the same essential amino acids (and in similar proportions) were in excess or deficit across all plants. Plants appeared to be notably deficient in the sulphur amino acids methionine, cystine and arginine.

Multivariate ANOVAs revealed a significant habitat effect on the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotopic patterns of blood plasma ($V = 0.53$, $F_{2,31} = 17.8$, $P < 0.001$) and blood cells ($V = 0.34$, $F_{2,27} = 6.8$, $P = 0.004$). Isotopic signatures of droppings differed between pasture and salt marsh in both $\delta^{15}\text{N}$ ($t = 5.8$, $df = 34$, $P < 0.001$) and $\delta^{13}\text{C}$ ($t = -3.7$, $df = 34$, $P = 0.001$), which further allows estimation of dietary input from these habitats (Fig. 2). In line with the results from multivariate ANOVA, estimates from mixing models revealed a higher dietary input from the habitat of capture (staging) than from the alternative staging habitat (Table 1), indicating a significant degree of faithfulness to one of the two habitats despite their close proximity. This was especially evident for blood plasma, but also for blood cells of geese caught in salt marsh. The estimated median contribution of pasture forage in plasma of geese caught in pasture land on 1 and 20 April was 61% and 85%, respectively, but 42% for geese caught in the salt marsh on 14 April. However, the results also suggest large variation within groups of geese and substantial mixing of diet, presumably originating from both habitats. A comparison of estimates inferred from blood cells and plasma suggest that 'pasture geese' used pasture forage mainly recently but have used more salt marsh forage over the one to two months before capture (Table 1).

A multivariate ANCOVA model including 'habitat', 'structural size' (PC1) and 'date' (March days) as explanatory variables revealed no significant effect of habitat on fat-free mass (FFM) and fat mass (FM) ($V = 0.04$, $F_{2,49} = 1.0$, $P = 0.4$), whereas both structural size ($V = 0.38$, $F_{2,49} = 15.0$, $P < 0.001$) and date ($V = 0.58$, $F_{2,49} = 34.1$, $P < 0.001$) were significant. For those individuals for which we had blood-isotopic data we conducted a multivariate ANCOVA where we replaced 'habitat' with its estimated median proportion of dietary input from pasture calculated from MixSIR models, while retaining 'date' and 'structural size'. Neither variation in dietary input from pasture estimated for blood

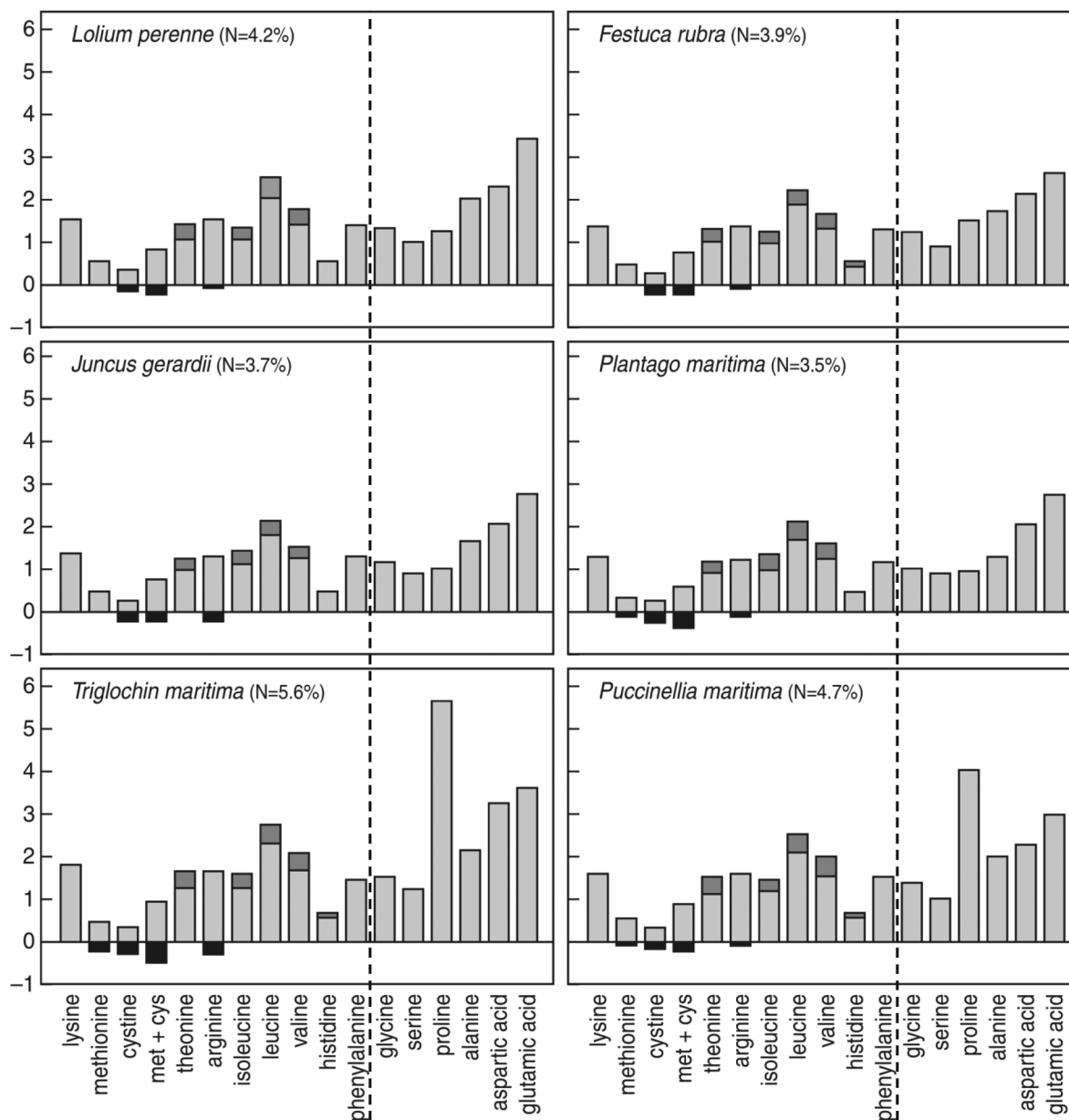


Fig. 1. Amino acid contents (% dry weight) in six plant species from the study area (*Lolium perenne* from pasture, all others from salt marsh) are shown by positive values. The first 11 columns from left to right (up to the vertical dashed line) refer to essential amino acids, the last 6 columns to non-essential amino acids. The measured amino acid contents are contrasted to ideal ratios of essential amino acid established for the growing chicken (see methods; no ideal ratio value was available for phenylalanine): proportions in excess of the ideal pattern are shown in dark grey; proportions in deficit are given as negative values in black. Columns for all non-essential amino acids and phenylalanine refer to the measured contents only. Total nitrogen (N) contents are given in brackets next to the plant species name.

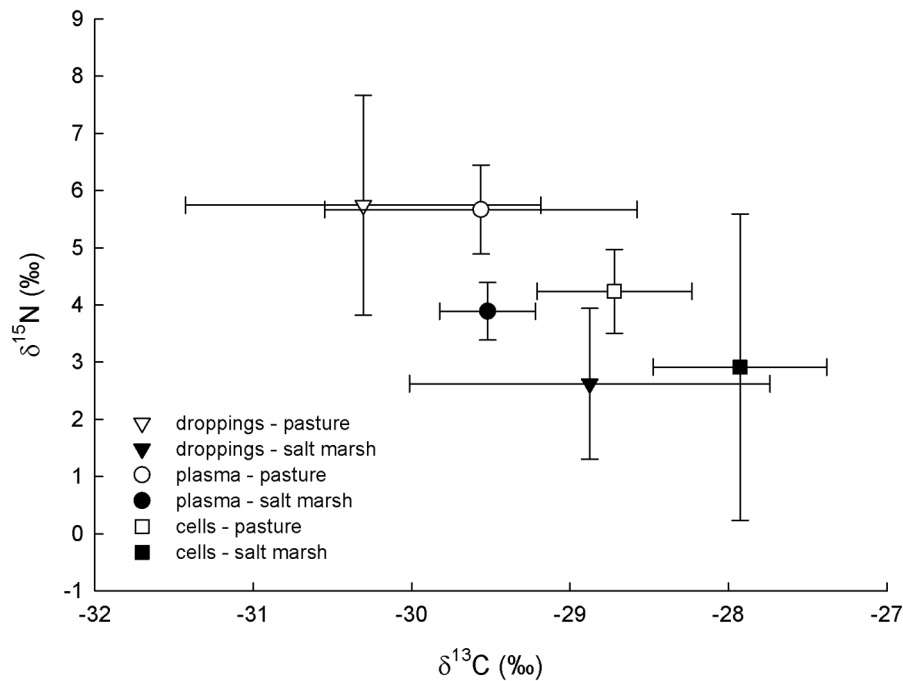


Fig. 2. Isotopic $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures (mean \pm SD) of goose droppings, blood plasma and blood cells sampled from barnacle geese staging in pasture (open symbols) and salt marsh habitat (filled symbols) on the Dutch island Schiermonnikoog. Blood values are adjusted for diet-tissue fractionation (see *Methods*).

cells ($V = 0.04$, $F_{2,26} = 0.5$, $P = 0.6$) nor for blood plasma ($V = 0.01$, $F_{2,22} = 0.1$, $P = 0.9$) had a significant effect on FFM and FM, despite large variation among individuals (Fig. 3).

As expected from multivariate tests, habitat had no effect on body mass (BM), FFM or FM in univariate ANCOVA tests (Table 2). Structural size explained significant variation in BM and in FFM but not in FM. Body mass in the total sample and FFM and FM in the sub-sample

increased linearly over the study period at rates of 10.5, 3.6, and 7.4 g/d, respectively (Table 2, Fig. 4). Accordingly, wet protein and lipids contributed 33% and 67%, respectively, to the body stores deposited by female barnacle geese.

DISCUSSION

Amino acids of food plants

The six main forage plants showed great similarity in their amino acid profiles. *Festuca*,

Table 1. Estimated contributions of pasture forage to the diet of barnacle geese caught in pasture or salt marsh on three occasions as inferred from stable isotope signatures in blood plasma and blood cells. Presented are the median contributions and, in brackets, the 5th and 95th percentiles as estimated from MixSIR models. Contributions of salt marsh forage can be calculated as one minus the respective diet proportion from pasture.

Blood tissue	N	Habitat of capture	Date of capture	Diet proportion from pasture
Plasma	8	Salt marsh	14 April	0.42 (0.27–0.58)
Plasma	11	Pasture	1 April	0.61 (0.49–0.75)
Plasma	15	Pasture	20 April	0.85 (0.72–0.97)
Cells	7	Salt marsh	14 April	0.19 (0.04–0.35)
Cells	11	Pasture	1 April	0.34 (0.19–0.49)
Cells	12	Pasture	20 April	0.36 (0.23–0.48)

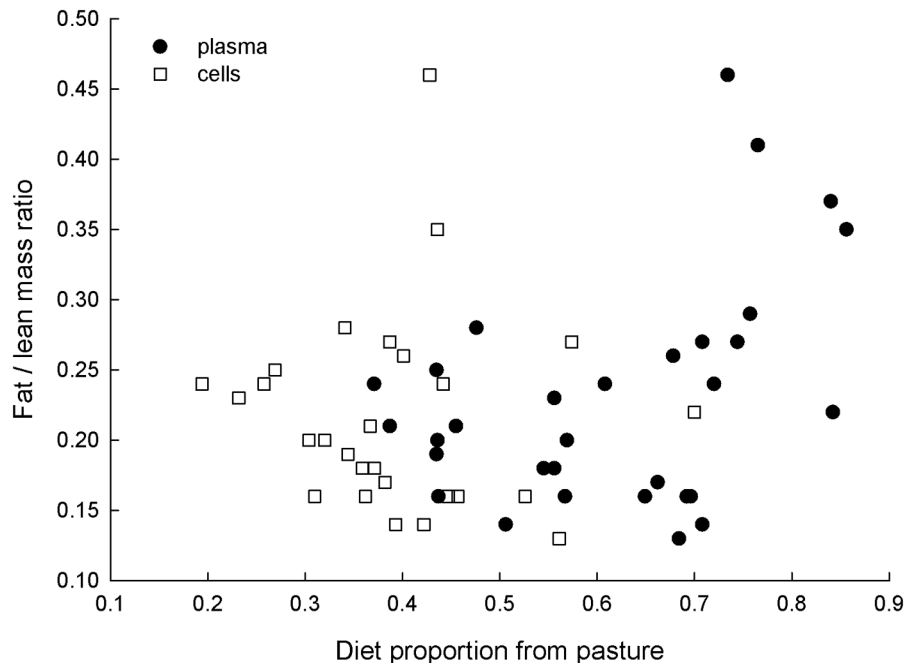


Fig. 3. Body composition and food habitat use in spring staging barnacle geese. Fat to lean mass ratios of individual geese are plotted against dietary contributions of pasture forage estimated from blood plasma and blood cells by MixSIR models (i.e., the median posterior source contributions are plotted).

as the main diet of barnacle geese in the salt marsh, was particularly similar to the pasture plant *Lolium*, both in absolute content and relative balance of amino acids. Also Sedinger (1984) found similar amino acid patterns among various goose forage plants from tundra vegetation in Arctic breeding grounds, with relative proportions of specific amino acids remaining constant over the season despite seasonal variation in total protein. He emphasized that 50–80% of leaf protein is in the form of a single enzyme, ribulose biphosphate carboxylase (Rubisco), which may explain the relative invariance of amino acid ratios. In the present study, proline was a notable exception to the relative invariance in plant amino acid content, with highest levels in salt marsh food plants. *Triglochin* and *Puccinellia* are known for their high accumulation of proline, which functions as osmotic solute in these halophytes (Stewart and Lee 1974, Jefferies 1980). Conceivably, these high levels of (dietary non-essential) proline are in excess of the goose's requirements. Therefore, actual food quality in terms of essential or limiting amino acid content will be smaller than estimated from the higher

total nitrogen content alone. For instance, *Triglochin* and *Puccinellia* had a, respectively, 33% and 11% higher total nitrogen content than the agricultural grass *Lolium*, but the content of lysine (the first limiting amino acid) was elevated by, respectively, only 23% and 5% (Fig. 1). Nevertheless, lysine content was highest in *Triglochin*, a food plant known to be highly favored and quickly depleted by brent geese (Prop and Deerenberg 1991).

All food plants generally showed a favorable balance, with the exception of sulphur amino acids. The relative demand of sulphur amino acids in adult geese out of molt, however, is likely to be lower than in growing chicken (on which the ideal amino acid profile is based) which have to synthesize much feather keratins. Elsewhere, geese also frequently feed on various grains (McLandress and Raveling 1981, Madsen 1985, Alisauskas and Ankney 1992). Although generally a better energy source their protein content is lower and deficient in certain essential amino acids, particularly lysine and methionine (McDonald et al. 1995). Therefore, geese relying on grain might be more vulnerable to encounter

Table 2. ANCOVA results of body mass (A), fat-free mass (B), and fat mass (C) of adult female barnacle geese during spring staging in pasture and salt marsh habitat. "Size" refers to the first principal component from a PCA of tarsus, head and wing length. Estimated coefficients (*b*) with associated standard errors (SE) are given only for statistical significant parameters (n.s. = not significant). The *F*-value of any n.s. parameter (except the intercept) refers to the value when this parameter was included in the final model. Variable "date" refers to March days with the intercept set at March day = 0.

Parameter	<i>b</i>	SE (<i>b</i>)	df	<i>F</i>	<i>P</i>
A) Body mass (g)					
Intercept	1395	26.3	1	2822	<0.001
Size	71.7	10.1	1	51.9	<0.001
Date	10.5	0.8	1	186	<0.001
Date ²			1	0.4	n.s.
Habitat			1	0.5	n.s.
Date × Habitat			1	1.0	n.s.
Final model ($R^2 = 0.70$)			2	126	<0.001
Total			108		
B) Fat-free mass (g)					
Intercept	1342	30.1	1	1984	<0.001
Size	62.1	10.9	1	32.2	<0.001
Date	3.6	0.8	1	21.1	<0.001
Date ²			1	1.9	n.s.
Habitat			1	0.02	n.s.
Date × Habitat			1	1.5	n.s.
Final model ($R^2 = 0.56$)			2	31.9	0.001
Total			53		
C) Fat mass (g)					
Intercept	30.4	36.4	1	0.7	n.s.
Size			1	0.3	n.s.
Date	7.4	0.9	1	61.4	<0.001
Date ²			1	1.2	n.s.
Habitat			1	2.3	n.s.
Date × Habitat			1	0.6	n.s.
Final model ($R^2 = 0.54$)			1	61.4	<0.001
Total			53		

problems in accumulating sufficient protein stores than those foraging on grass leaves.

Habitat use and the deposition of body stores

Our plasma stable isotope findings are in line with previously found high site fidelity in barnacle geese (Van der Jeugd et al. 2001, Bos and Stahl 2003), within a one to two week time frame. Over a term of several weeks, however, this site-fidelity is less pronounced as indicated by the isotopic signatures of blood cells. This reduced long-term site fidelity probably reduces our ability to detect potential habitat effects when using site of capture as a proxy for habitat choice. Importantly, however, we also found no effect on body composition when using isotope mixing

models instead of habitat of capture in assigning habitat use until capture.

The present study confirms the notion that long-distance migrants such as the barnacle goose accumulate both protein and fat stores during spring staging. These nutrient stores are needed to fuel migratory flight (Jenni and Jenni-Eiermann 1998) and contribute to egg formation and incubation (Drent et al. 2007, Hahn et al. 2011). There are three approaches to discriminate between fat and protein: (1) carcass analysis, (2) isotope dilution and (3) field studies on nutrient budgets and digestibility. We employed method (2) and found no difference in the body composition of barnacle geese utilizing *Lolium* pasture as compared to salt marsh. Wet protein comprised a third of deposited body stores during the one-month study period of premigratory fuelling, which translates into a fat to protein ratio of 8.1 (assuming 75% water content in protein, Eichhorn 2008). Prop and Black (1998) applied method 3 for barnacle geese of the Spitsbergen population at a late spring staging site in Norway covering events in early May (2–3 weeks), and give an overview of results achieved for other goose species by method 1. Prop and Black (1998) estimated fat to dry protein ratios of stores of 1.2 to 1.4 for geese staging in salt marshes (feeding mainly on *Festuca rubra*), which contrasted strongly with a ratio of 22.6 estimated for geese feeding on improved agricultural grassland (of *Phleum* and *Poa* spp.). Employing the same method 3 for a study on spring staging brent geese in the Dutch Wadden Sea, Prop and Spaans (2004) found again higher fat but lower protein deposition rates for geese using intensively managed pasture (of *Lolium perenne* and *Poa* spp.) versus salt marsh (noteworthy, their salt marsh study site being the same as in the present study).

In both studies Prop and co-workers found lower nitrogen retention efficiencies for geese feeding on agricultural grass, which was not related to a depressed assimilation of nitrogen in the food but to a higher loss of urinary nitrogen. When we analyzed nitrogen partition in barnacle goose droppings from the present study we found a similar pattern: total nitrogen content in droppings was considerably higher for geese feeding on pasture (58 ± 2.4 g/kg dry weight; mean \pm SE, $N = 8$) compared to salt marsh ($43 \pm$

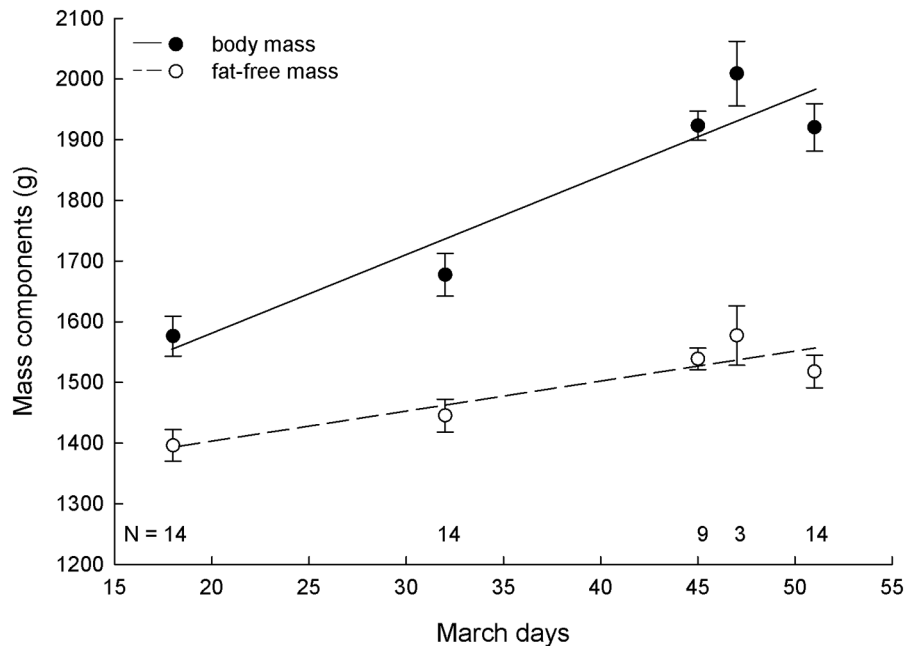


Fig. 4. Development of body mass components (means \pm SE) in adult female barnacle geese during spring staging on the Dutch island Schiermonnikoog. Changes in total body mass (filled circles, solid line) or fat-free mass (open circles, broken line) did not differ between geese from pasture or salt marsh habitat (Table 2). Rates of mass increase are 3.6 g/d for fat-free mass and 10.9 g/d for body mass ($N = 54$; 10.5 g/d if body mass in the total sample of $N = 109$ birds is modeled; Table 2). Sample sizes are given at the bottom of the figure.

1.9 g/kg; $N = 8$), and the difference concerned mainly nitrogen from urinary waste products (47 ± 2.2 g/kg pasture; 34 ± 1.7 g/kg marsh) and not fecal nitrogen (12 ± 0.4 g/kg pasture; 9 ± 0.4 g/kg marsh). The result that pasture geese make less use of the apparently higher protein content led Prop and co-workers to the suggestion of a less favorable amino acid composition of agricultural grass.

Estimating protein deposition from nitrogen budgets is complicated because of the various sources of nitrogen that are usually not accounted for. One assumption in these budgets is that food nitrogen (N) reflects crude protein ($CP = N \times 6.25$). Deviations from this assumption can be large, especially in plant matter. For instance, Sedinger (1984) found that crude protein overestimates protein content by as much as 22–52% in goose forage plants of tundra vegetation. Furthermore, all assimilated food nitrogen that is not retained by the body is assumed to represent excess amino acids which are, after deamination, catabolized for energy or used to synthesize fatty

acids for energy storage. However, nitrogen in forage comes, apart from proteins, from a variety of non-protein nitrogen sources including free amino acids, nucleic acids, amides (e.g., urea, uric acid), amines, nitrates and further nitrogenous compounds (McDonald et al. 1995). Free amino acids, which can be present in substantial amounts in certain halophytes, will be readily used for the building of body proteins or, if in excess, catabolized for energy. But other non-protein nitrogen compounds can neither be used for protein building nor for energy and will enlarge the amount of urinary nitrogen. Due to the intensive application of fertilizers many of these nitrogenous compounds may be particularly present in agricultural grassland. This is clearly the case for at least nitrate, which occurred with 0.22 ± 0.05 (mean \pm SE, $N = 4$) and 0.12 ± 0.01 ($N = 3$) g/kg dry weight, respectively, in the salt marsh plants *Festuca rubra* and *Juncus gerardi*, but 30 to 56 times more, with 6.7 ± 1.5 g/kg ($N = 4$) in the pasture plant *Lolium perenne*. Nitrate nitrogen made up only

2.5% of the total nitrogen in *Lolium*. But further nitrogen sources in fertilizers, especially amides and amines, will add to the pool of non-protein nitrogen in pasture forage and altogether may cause considerable deviation of estimated crude protein content from true protein content. Thus, if these compounds are present in significant amounts, total food nitrogen should be corrected accordingly in order to calculate true protein retention efficiencies from nitrogen balances. If not accounted for, protein intake will be overestimated and protein retention efficiency underestimated accordingly.

Population increase, shift in habitat and migratory behavior

Since the 1960s the East Atlantic Flyway population of the barnacle goose has increased more than twenty-fold to an estimated 550,000 individuals in 2006. Over the past four decades this species has colonized new breeding sites to the southwest of the original breeding grounds in arctic Russia, including colonies within the wintering area along the North Sea coast (Van der Jeugd et al. 2009, Eichhorn et al. 2010). Moreover, over the past two decades an increasing number has delayed departure from the North Sea extending spring staging by up to one month, probably driven by higher energy gain and lower predation rates compared to staging sites in the Baltic (Eichhorn et al. 2009, Jonker et al. 2010). A comparison of the rate of BM gain of female barnacle geese from the present study (10.5 g/d) with that estimated from data collected during March-April in 1979 and 1989 (3.8 g/d) points also to an improvement in absolute fuelling rates in the North Sea over past decades, though BM early in the season was higher in former times presumably related to the earlier departure (Eichhorn 2008). Altogether, the North Sea area, particularly through its agricultural pastures, has gained significantly in importance as fuelling site for barnacle geese and other herbivorous waterbirds (Van Eerden et al. 2005) with concomitant increased conflict between geese and farmers.

Conclusions

The present study revealed no evidence of impaired protein accretion in geese using intensively managed pasture compared to natural salt

marsh. Forage from pasture appeared comparable to forage from salt marsh with respect to overall content and composition of amino acids. Body composition of barnacle geese appeared not to be affected by the degree to which they utilize pasture forage. While endogenous nitrogen budgets offer the least invasive approach for the estimation of protein deposition in wild animals, results are prone to varying levels of food non-protein nitrogen unless accounted for. It will require detailed studies on the partitioning of nitrogenous sources in those budgets to assess the full significance of this problem and to explain the contrasting nitrogen retention efficiencies of geese feeding in pasture and salt marsh. Currently, geese all over the world seem to benefit from the exploitation of agricultural foods, a favorable development that allowed them to extend their naturally limited feeding habitat. However, the continuing man-made loss of their natural habitat and increasing dependence on agricultural crops renders geese 'captive of agricultural policy' (Jefferies and Drent 2006). Such dependency combined with possible changes to crops not favorable to geese may quickly turn the current boon into a future bane.

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